

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Peter R BRINK <i>et al.</i>	Confirmation No.:	9822
Application No.:	10/584,303	Examiner:	Ha, Julie
Filed:	April 5, 2007	Group Art Unit:	1654

For: CREATION OF A BIOLOGICAL ATRIOVENTRICULAR BYPASS TO
COMPENSATE FOR ATRIOVENTRICULAR BLOCK

Commissioner for Patents
Alexandria, VA 22313-1450

AFFIDAVIT UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Michael R. Rosen, M.D. hereby attest to the following:

1. I am the Pfeiffer Professor of Pharmacology and Pediatrics; Director, Center for Molecular Therapeutics, Presbyterian Hospital, Room 7 W 321, 622 West 168th St., New York, NY 1003.
2. I am the co-inventor of the inventions disclosed in US Patent Application 10/584,303, filed on April 5, 2007, Brink et al., which claims priority to priority from, U.S. Provisional Application No. 60/532,363, filed Dec. 24, 2003.
3. I am also co-inventor of the inventions disclosed in US Patent Application 10/342,506, 20040137621, filed on January 15, 2003, Rosen et al.
4. I hereby declare that any invention disclosed but not claimed in Rosen, et al., US Patent Application 10/342,506, was derived from me and is thus not an invention "by another" with respect to Brink et al., US Patent Application 10/584303.
5. The atrioventricular bypass tract (AV tract) of the present invention is formed in culture and is a functioning, conducting syncytium even before it is implanted in the heart, which is an important advancement over the prior art and key difference from Taheri et al., U.S. Patent 6690970 (Taheri). Figure 2 of Brink et al. shows that the human MSCs cultured

in vitro form gap junctions with one another and that gap junction currents (I_j) passed between the connected cells.

[0014] FIG. 2. Macroscopic and single channel properties of gap junctions between hMSC pairs. Gap junction currents (I_j) elicited from hMSCs using symmetrical bipolar pulse protocol showed two types of voltage dependent current deactivation: (A)-symmetrical. (B): asymmetrical.

The hMSCs are also able to couple *in vitro* electrically with each other, with HeLa cells that express connexin 43, and with canine ventricular cells (Figs. 3 and 4, respectively). See also Paragraph [0034]. This means that the strip is a functional syncytium that can be tested for the ability to conduct current before implantation.

By contrast Taheri only describes injecting individual cells into a specific area of the heart where a conductance blockage has been identified. Once injected, the cells STILL grow *in situ* to form gap junctions with one another in sufficient numbers to form a syncytium that can conduct signals around the blockage. Nowhere does Taheri describe growing a “strip” or fully functional syncytium of conductive cells *in vitro*, for subsequent implantation in the heart.

A further advantage of the present invention is that implanting a functional syncytium (the strip) into the heart will return conductivity to the heart within a significantly shorter time, as opposed to implanting cells that have to “grow” *in situ* to form a conductive bridge.

6. Submitted with this Declaration is evidence that there is a 6-fold higher delivery of hMSCs to the heart when the hMSCs are bound to a nonbioreactive material and implanted into the heart, as opposed to being injected in a solution as free cells. The nonreactive biomaterial used to make a strip of hMSCs for implantation in a heart was made by bundling 8 fibrin microthreads with 4 collagen microthreads and attaching this bundle to a suture needle. This bundle is hereafter referred to simply as “microthreads.” The microthreads were seeded *in vitro* with 100 μ L of cell suspension of human mesenchymal stem cells (about 100,000 hMSCs). After one day in culture, the microthreads were bound to an average of $11,692 \pm 2,211$ hMSCs. The microthreads bearing the hMSCs were attached to suture needles to allow targeted delivery to the left

ventricular wall of the rat heart. For intramyocardial injection, a cell suspension containing 10,000 FREE hMSCs (35 μ L) was injected into the myocardial wall using a 100 μ L syringe. The delivery efficiency of each method was determined by sectioning the heart into 8 μ m thick sections and analyzing three sections every sixty sections for quantum dot loaded hMSCs.

One of the main objectives of this study, done by a graduate student of one of our collaborators, was to maximize the delivery of hMSCs to the left ventricular wall in the rat heart. All experiments were histologically analyzed and the hMSC delivery distribution and efficiency were determined. The biological microthread implantation group ($n = 5$) had a delivery efficiency of $66.6 \pm 11.1\%$, which is 6-fold higher than that of the intramyocardial injection group ($n = 4$) that had a delivery efficiency of $11.8 \pm 6.25\%$. Thus, biological microthread implantation was significantly more efficient ($p < 0.05$, see Figure 19). The total number of cells engrafted in each group was calculated (see Figure 20). The average number of cells engrafted for each biological microthread implantation was $3,527 \pm 624$, while the average number of cells engrafted for each intramyocardial injection was only $1,146 \pm 490$. Microthread implantation delivered significantly more hMSCs ($p < 0.05$), indicating that hMSC-seeded microthreads were successfully delivered to the selected target area of the left ventricle, and hMSCs were uniformly distributed along the length of the implanted microthread (after an initial increase in the number of cells delivered). This study illustrates that microthreads are an efficient means of delivering hMSCs to the heart, and that implantation of cells in a strip, such as cells bound to a nonreactive biomatrix, is much more efficient for delivering cells to the heart than injecting individual cells. This work is described in detail in *Delivering Stem Cells to the Heart*, Master's Thesis, Worcester Polytechnic Institute, by Michael Fakharzadeh, a copy of which is submitted with this Declaration.

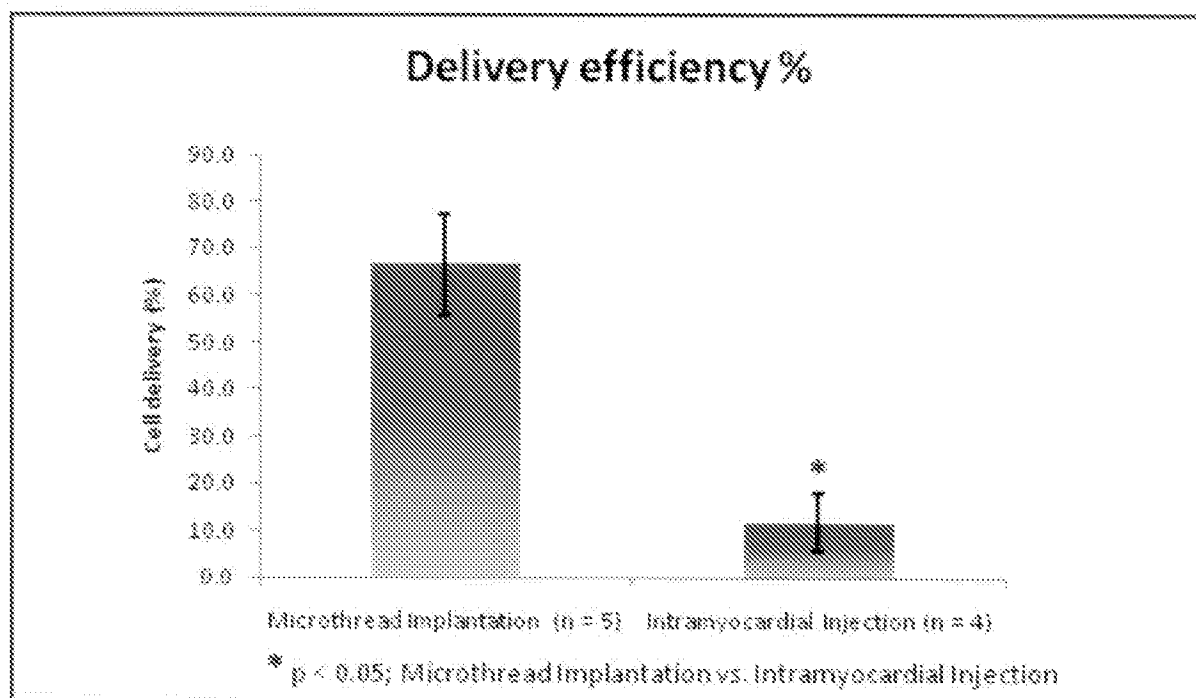


Figure 19: hMSC delivery efficiency comparison (%): microthread implantation vs. intramyocardial injection

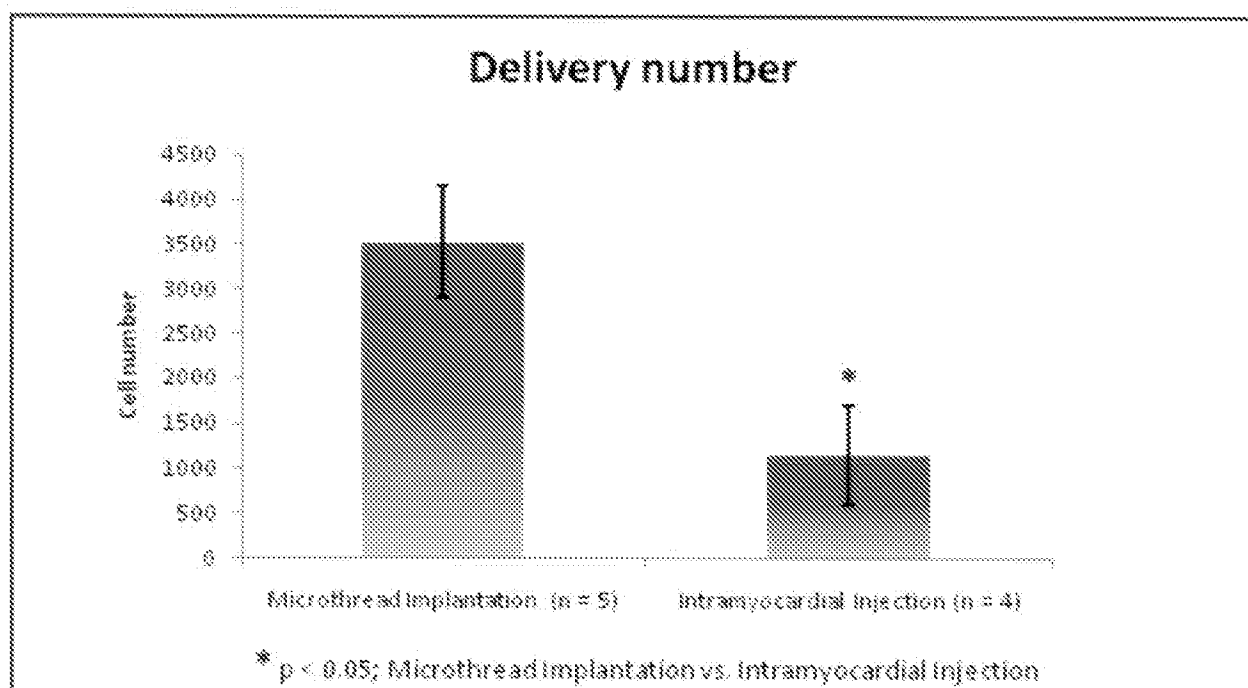
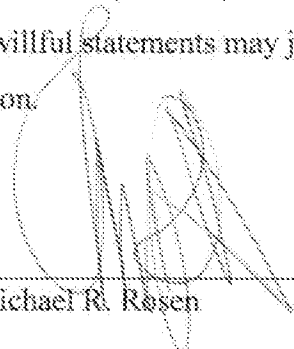


Figure 20: hMSC delivery number comparison: microthread implantation vs. intramyocardial injection

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

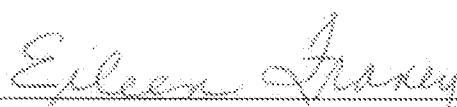
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Date



Michael R. Rosen

The signature of Michael R. Rosen is witnessed by:

11/23/10
Date



Signature

Name: EILEEN FRANEY